HAND BOOK ON
BREEDING AND SEED PRODUCTION OF COBIA AND POMPANO

Central Marine Fisheries Research Institute
Mandapam Regional Centre
Mandapam Camp - 623 520
Tamil Nadu, India

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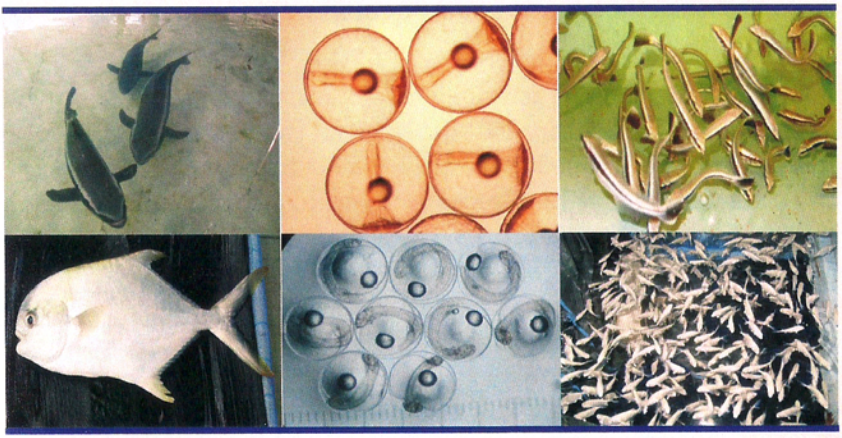

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PREFACE

In recent years finfish mariculture has been growing rapidly on a global basis especially with the development and expansion of sea cage farming. One of the major reasons for the growth of sea cage farming is the availability of breeding techniques that can produce sufficient quantity of seeds of different high value marine finfish. Many countries in the Asia-Pacific Region like Australia, China, Japan, Taiwan, Philippines, Indonesia, Thailand, Malaysia and Vietnam have made substantial progress in the development of commercial level seed production technologies of many high value finfish suitable for sea farming. But even in these countries, seed stock supply is one of the vital issues for further expansion of mariculture.

In India, much research attention was not given for developing seed production methods for high value finfishes suited for sea farming. At present we have commercial seed production of only one marine finfish – sea bass (*Lates calcarifer*). Here also private entrepreneurship has not yet been developed. The vital issue for the expansion and commercialization of sea farming in India is the availability of suitable seeds of high value finfishes. Unless an intensified research and development effort is given to the development of commercial level seed production technologies, sea farming cannot emerge as a significant seafood production sector in the country. In the recent past, the Central Marine Fisheries Research Institute (CMFRI) has been intensifying its research activities on the breeding and seed production of high value marine finfish and success was achieved in the breeding and seed production of cobia and silver pompano for the first time in the country at Mandapam Regional Centre of CMFRI.

Cobia (*Rachycentron canadum*) and silver pompano (*Trachinotus blochii*) are two marine finfish species with very high potential for aquaculture in India. Fast growth rate, adaptability for captive breeding, lowest cost of production, good meat quality and high market demand especially for *sashimi* industry are some of the attributes that make cobia an excellent species for aquaculture. In recent years the seed production and farming of cobia is rapidly gaining momentum in many Asian countries. Envisaging the prospects of cobia farming in India,

broodstock development was initiated at the Mandapam Regional Centre of Central Marine Fisheries Research Institute in sea cages during 2008 and the first successful induced breeding and seed production was achieved in March – April 2010. Trials on sea cage farming carried out at Mandapam showed that the fishes attained an average weight of 2.5 kg in six months and 7.3 kg in twelve months. The species can be grown in low salinity and experiments revealed that up to 15ppt the growth and survival is comparable to that in seawater. These results point out the possibility of developing a lucrative cobia aquaculture enterprise in the country. Similarly among the many high value marine tropical finfish that could be farmed in India, the silver pompano is also one of the topmost, mainly due to its fast growth rate, good meat quality and high market demand. The species is able to acclimatize and grow well even at a lower salinity of about 10 ppt and hence is suitable for farming in the vast low saline waters of our country besides its potential for sea cage farming. At Mandapam Regional Centre of CMFRI, successful broodstock development, induction of spawning and fingering production of silver pompano was achieved during July 2011 for the first time in India. Subsequently four more seed production experiments were also done successfully and now farming trials are progressing. This can be considered as a milestone towards the development of pompano aquaculture in the country. Ever since the achievement was made by CMFRI on the seed production of cobia and pompano, there has been an increasing demand for the seed from the coastal fish farmers of the country. A few farmers were supplied with the hatchery produced seed and the farming results are encouraging. In this context it was felt that supplying the seed to meet the growing demand from all the entrepreneurs by the Institute may not be a practical proposition. Hence transferring the technology through training was considered as the best option. A brief manual was necessitated for this purpose. It is felt that this condensed manual will be helpful for getting a firsthand information on the vital aspects needed on the techniques involved in marine finfish breeding to the hatchery technicians.

Mandapam Camp,
15-2-2012

(G.Gopakumar)
Head, Mariculture Division

CONTENTS

1. Introduction

The major steps involved in cobia and pompano breeding and seed production are the following:-

- Broodstock collection
- Transportation
- Quarantining
- Broodstock development
- PIT tagging
- Cannulation
- Induction of spawning
- Egg collection
- Incubation
- Larval feeding
- Grading
- Fingerling production
- Nursery rearing
- Micro-algal culture
- Greenwater technique
- Rotifer culture
- Artemia hatching
- Copepod culture
- Nutritional enrichment of live feeds

2. Broodstock

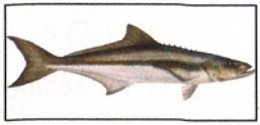
Broodstock Collection and handling

Broodstock fish are generally collected from the wild and are conditioned and matured in captivity. The main selection criteria to identify suitable adult fish as broodstock fishes are size, age (for those collected from grow-out farms) and appearance. The following are the details of the selection criteria:-

- *body shape, age and colour,*
- *absence of deformities,*
- *absence of wounds, haemorrhages, infections and parasites,*
- *behaviours like quick response to feed and fast swimming*

It is advantageous to collect sub-adults for broodstock development. Larger fishes would have crossed the reproductive age & very small fishes will take longer time to sexually mature.

Collect the cobia weighing between 8 to 15 kg for broodstock development. Whereas, the pompano brooders could be procured in the weight range of 750 g to 1.5 kg.



Cobia brooder

Stress should always be minimised during capturing and handling of broodstock. It is best to collect broodstock fishes from trap nets, hook & line, *etc.,* as they cause minimum stress to the fishes. Adequate dissolved Oxygen (DO) should be ensured during transportation.

Pompano brooder

Quarantine treatment

Upon arrival at the hatchery, broodstock fishes are released into the quarantine tanks for prophylactic treatment. Fish Anaesthetics like MS 222 (50-100 ppm) and Aqui-S (4 ml / 100 L), can be used for broodstock handling. The prophylactic treatment is given to limit the risk of introducing parasites or bacterial diseases into the hatchery facility. Short time exposure of brooders (5 – 15 minutes) in freshwater

will help to remove the external parasites. The prophylactic treatment in hatcheries includes a sequence of medicated baths in formalin, malachite green and Oxytetracycline (OTC). Prophylactic treatment can be repeated three to four times within a week.

It is preferable to have a flow-through water circulation in quarantine tanks when treatments are not underway. Smooth inner surface in tanks allow easy and complete cleaning.

Following sequence of treatments can be followed:-

Day 1: Fresh water bath for 10 minutes and then Oxytetracycline treatment (50 ppm) in seawater for 30 minutes.

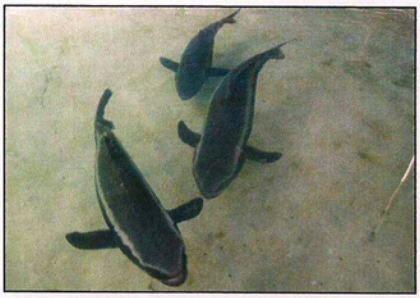
Day 2 to Day 7: Treatment with a mix of 200 ppm formalin and 0.2 ppm malachite green for 1-2 minutes, followed by a freshwater dip for 5 minutes. Before returning the fishes to quarantine tanks with filtered sseawater, they can be given an Oxytetracycline treatment at 50 ppm for 30 minutes.

During the quarantine, fish should be closely monitored and fed during the day time when it is not undergoing treatment. Over feeding should be avoided and the fishes can be transferred to maturation tanks after the treatments are over.

Apart from quarantine treatment, the broodstock fishes should be given regular prophylactic treatment with freshwater with or without OTC at least once in a month.

Broodstock holding and maturation

After quarantine, broodstock fishes are moved into 100 tonne capacity RCC tanks for maturation and long-term holding in the hatchery. During gonadal maturation, water salinity needs to be 31-35 ppt. Water quality parameters like salinity, temperature, dissolved oxygen, pH, ammonia, and fish stock condition *viz.*, general behaviour, feeding activity, disease symptoms, prophylactic treatments, etc. are monitored regularly. Normally sex ratio of 1 female: 2 males are maintained for cobia while it is 1: 3 for pompano.



Cobia broodstock fishes

Broodstock tank photos

Broodstock development in cages

For larger fishes like cobia, broodstock development in FRP tanks/ RCC tanks is possible only with recirculating aquaculture system due to its high metabolic rate. Alternatively, broodstock development can more effectively be practised in circular (6 meter diameter and 4.5 meter net cage length) or square (5 m X 5m) sea cages.

Broodstock cages at Mandapam

Broodstock Feeding

For quicker maturation, the broodstock fishes are to be fed with highly nutritive diet. Diet rich in vitamins, poly-unsaturated fatty acids (n- 3 PUFA) and other micro-nutrients is essential for obtaining viable eggs and larvae. During gametogenesis female fish require a food, richer than usual, in proteins and lipids to produce the vitellogenin, which is progressively stored as yolk in the oocytes. As the sole source of food for the developing embryo and the early larval stage until feeding on live preys starts, yolk quality and quantity are key factors for a

Artificial Broodstock feed

Trash fish

Squid

successful reproduction. Both dry pellets and moist food are also employed during maturation. Dry pellets should include essential nutritional components like polyunsaturated fatty acids (n-3 PUFA), in particular EPA (20:5 ω 3) and DHA (20:6 ω 3), which cannot be produced by fish metabolism . Broodstock fishes are fed *ad libitum* once a day with chopped oil-sardines, crabs, shrimps and squids depending on the availability.

3. Tagging of Fish

Tagging or physical marking of broodstock fishes through easily detectable methods is very much essential for selection of broodstock for identification, selective breeding and segregation. The most popular method is PIT Tagging.

What is PIT tag?

Passive Integrated Transponder (PIT) tag, also known as 'microchips' is a radio frequency device to permanently mark fishes internally. The tag is designed to last the life of the fishses providing a reliable, long term identification method.

Principle of PIT tag

The PIT tag contains a microprocessor chip and antenna. It has no internal battery, hence the term "passive", so the microchip remains inactive until read with a reader. The reader sends a low frequency signal to the microchip of the tag providing the power needed to send its unique code back to the reader and therefore fish is positively identified.

Read range and frequency of the tag

The distance from which a tag can be read is the read range. Most read ranges using hand-held readers are 3 to 9 inches depending on the reader.

There are currently three basic tag frequencies. The 400-kHz tag was one of the first developed but it has limited read range. As microchip technology evolved, the 125-kHz and 134.2-kHz tags became available. Compared to the older 400-kHz tags, they have a much better read

range and reduced read time. The 134.2-kHz tag was developed to meet international standards for code format. It is very much important that the tag type and reader unit should be compatible. Most readers are capable of detecting both 125-kHz and 134.2-kHz frequencies.

Tags can be read through materials such as soil, wood and water. Ferrous metals and noisy environments can cause interference between the electromagnetic communication of the reader and the tag.

Durability of the tag

Design engineers' calculations suggest that PIT tags can last as long as 75 years or more. There is no battery to fail and the glass encapsulation is impervious to almost everything. PIT tags can be removed or recovered from a primary location and reused indefinitely.

Safety to animal

Reducing stress to the fish is the prime factor in ensuring the success of the tagging and safety of the fish. Therefore, the fish should be anesthetized during the implantation of PIT tags. Species, size and age should be considered when making a decision about anesthetization and restraint. Sterile implants are advised but many field conditions do not allow for sterile implants. Equipment can be disinfected prior to use with alcohol and iodine-based solutions. The tag is encased in glass that protects the electronic components and prevents tissue irritation, thereby very much safe to the fish.

PIT tagging equipment

Tagging of cobia

Advantages of PIT tag over other tags

- Highly reliable individual identification
- Permanent identification marker
- Small size and no interference with the behaviour of fish
- No error in recording data
- Rapid data collection

Disadvantages

- Initial cost is high
- Low detection distance

PROCEDURE OF TAGGING

Site of implantation

The implant site depends upon the species, size of the fish and the size of the tag. It is preferable to implant the tag on the dorsal / ventral musculature of the fish which will be convenient for the brood fishes to be read.

Stepwise protocol

- Use sterile needle or implanter to tag the fish. In field condition, disinfect all the components prior to use with alcohol and iodine-based solutions.
- Read the tag before inserting into the fish and record the identification code or number.
- Catch the fish and anaesthetize it with suitable anaesthetic. In sea cages, it is easier to restrain the fish inside the catching net.
- Disinfect the site of implantation with alcohol or iodine-based solution.
- It is a better practice to keep a standard site of implantation so that the reading will be easier and quicker.
- The tag loaded inside the implanter has to be inserted into the muscle tissues. It is advisable to insert the tag parallel to the muscle fibres to avoid much damage to the tissues.

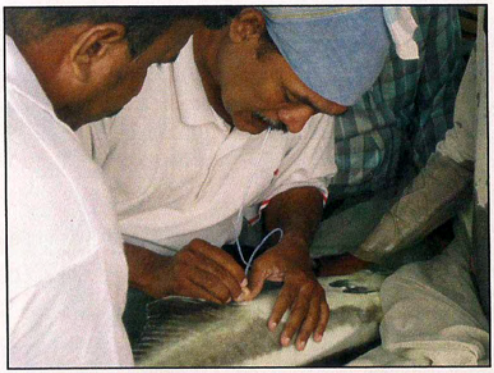
- The tag should be released slowly and steadily from the implanter while removing the implanter from the tissue in such a way that the tag fills the space created by the implanter needle.
- Once implanter needle is taken out, the site should be disinfected again with alcohol or iodine-based solutions to avoid secondary infection.
- Release the fish as soon as the tagging is over or once it has recovered from anaesthesia.

4. Maturation and spawning

The natural process of sexual maturation of the broodstock fishes can be accelerated by altering the photo-thermal period and it is also possible to obtain viable larvae almost throughout the year. At the onset of the spawning season, it is necessary to move selected broodstock fishes from maturation tank to spawning tank after assessing the ovarian development through cannulation. Only females with oocytes in the late-vitellogenic stage, with a diameter around 700μ in cobia and 500 μ in pompano, are selected.

Ovarian biopsy can be carried out as follows :

- Female brooders have to be transferred to a small tank containing anaesthesia in sufficient quantity
- Flexible sterile catheters (1.2 mm internal diameter) can be used for cannulation biopsy
- Introduce the sterile catheter into the oviduct, up to the ovary for a few cm; then suck carefully a small sample of oocytes up into the catheter and place the sample on a glass slide;
- After sampling, release the animal into the spawning tank, where recovery from sedation will take place;
- Put few drops of filtered sea water on the biopsy sample and examine under the microscope and measure the diameter of the oocytes and record the measurements.



Cannulation of cobia brooder

Induced spawning

Spawning can be obtained either naturaly or by inducing with hormones. Induced breeding is commonly practiced in most commercial hatcheries. The hormonal treatment is intended to trigger the last phases in egg maturation, i.e. a strong egg hydratation followed by their release. However, if eggs have not reached the late-vitellogenic (or post-vitellogenic) stage, the treatment does not work; hence ovarian biopsy is essential for assessing the ovarian development. The human chorionic gonadotropin (hCG) is used at a dosage of 500 IU per kg of body weight in cobia females and 250 IU per kg body weight for males, whereas, for pompano 350 IU per kg body weight is used for both male and female. This dosage can be administered as a single dose on the dorsal muscles. Use of hCG treatment sometimes gives serious setbacks like not all females respond to it, egg quality may be below acceptable standards with hatching rate lower than 80%, being a large molecule it may provoke immunization reaction , and as a result, fish treated with hCG may not respond when treated repeatedly with this hormone. However, hCG can be successfully replaced by an analogue of the luteinizing hormone-releasing hormone [LH-RHa des-Gly10 (D-Ala6) LH-RH ethylamide, acetate salt]. It is a small molecule with 10 peptides and acts on the pituitary gland to induce the release of gonadotropins which, in turn, act on the gonads. Almost 100% of injected fish spawn eggs whose quality usually matches that of natural spawning.

The cost of LHRHa is very high compared to that of hCG. But, LHRHa is used in very low dosages, usually around 20 µg / kg of body weight

Hormonal administration to cobia

Spawning tanks

The spawning unit should preferably be kept separated from the main hatchery building to avoid disturbance to the spawners and possible risk of disease contamination. However, for economic reasons, it is usual to keep the brooders inside the hatchery in a specific dedicated area. Though we use only rectangular tanks based on availability, it is preferable to use circular tanks with at least 1.20 m depth. Shape and depth counts for easy and free movement of brooders.

Normally the spawning could be noted within 36 -48 hours after hormonal induction. The spawning in cobia and pompano takes place normally between late night and early morning hours. The number of eggs spawned by cobia ranges from 0.4 to 2.5 million, whereas, the pompano brooders spawn 0.5 to 1.5 lakh eggs.

Egg harvest

The fertilized eggs of cobia and pompano float and are scooped gently using 500 µ net. To minimise the presence of poor-quality eggs, which usually float deeper in the water, it is advisable to collect only the eggs which float at the water surface. Therefore, aeration can be switched off allowing the unfertilized / dead eggs to settle at the bottom of the tank. The floating layer of eggs thicker than one cm

should be avoided. A thicker layer may reduce oxygen supply to the eggs, leading to possible anoxia after a short time. Then in the temporary container, eggs must be thoroughly examined to assess their quality, number and developmental stages. With a pipette eggs should be taken from the floating egg layer in the temporary container, and should be placed on a watch-glass or on a Petri dish for observation under microscope. Few dozens of eggs, which are placed under a microscope or a transmitted-light stereomicroscope have to be observed for the egg developmental stages.

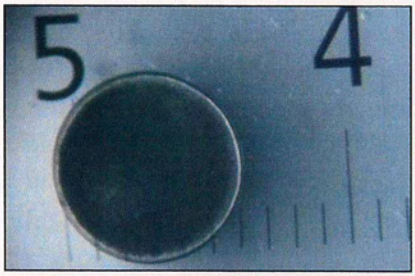
As fertilised cobia/ pompano eggs float in the seawater, they can be collected using egg collectors. If well dimensioned and properly placed, these devices harvest only the floating eggs, while the dead or unfertilised ones sink to the bottom. The presence of eggs in the collectors should be checked rather frequently in the case of cobia, as its spawning releases a large amount of eggs in a very short time there is risk of clogging the collectors or of mechanical stress to the eggs.

Check for the following egg characteristics:

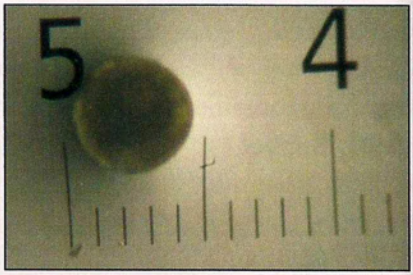
- presence of opaque, whitish eggs which are unfertilised. Similarly, eggs in the sample with transparent, but without evidence of cell divisions
- regular rounded shape and size (diameter 900-1000 µm in cobia; 800 -900 µm in pompano), regular cell division that can be observed only in the first blastomers; regular shape of yolk (it should occupy the egg volume entirely, without perivitelline space),
- absence of parasites or associated micro-organisms on the chorion surface.

Incubation of eggs

Incubation of eggs is done in incubation tanks of 3-5 tonne capacity. After hatching, only the hatched fish larvae have to be moved



Good quality egg



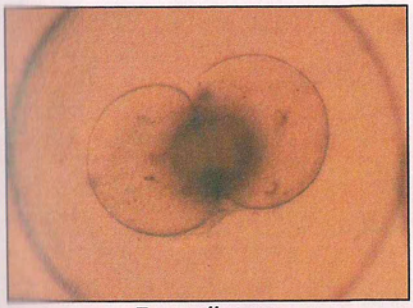
Under developed eggs

Unfertilized eggs

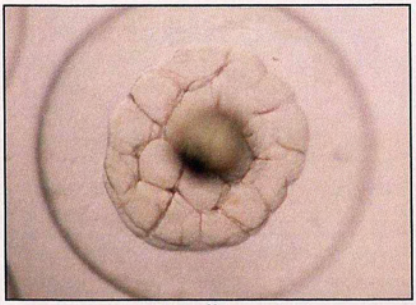
to the larval rearing tanks filled with filtered seawater. Prior to this, the aeration should be stopped briefly to enable the debris and exuviae to settle at the bottom which can be removed by siphoning.

Aeration needs to be adjusted suitably, not too strong to avoid excessive physical collision among eggs, but not too weak either, to keep the eggs suspended in water column. The main purpose of aeration is to prevent clumping and settling down of eggs. Air bubbles should not be too small as seen while using air diffusers instead of stones, as it results in clumped eggs and damage of the eggs. It is suggested to limit as much the number of air stones as possible. Stocking density can be maintained at a moderate level of 200 to 500 eggs per litre. The development of embryo can be observed at frequent intervals under a stereo / compound bionocular microscope. The hatching of eggs takes place from 18 to 24 hours

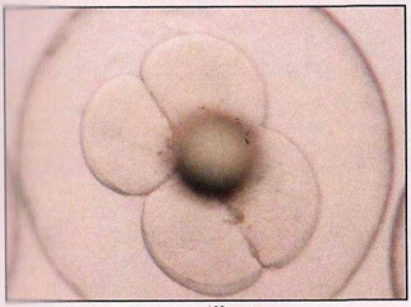
As the fecundity is normally high in cobia, we may require more incubation tanks, whereas the pompano requires only one tank /female



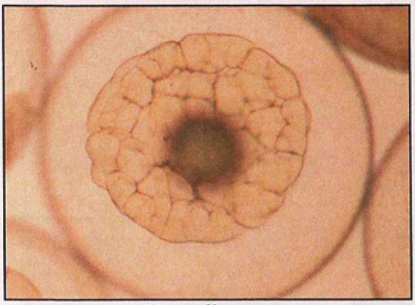
Two-cell stage



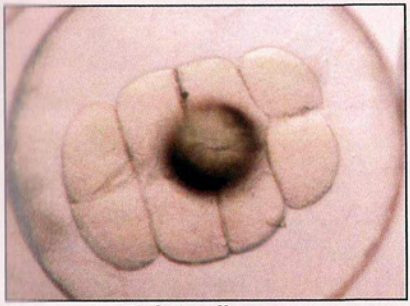
32 - cell stage



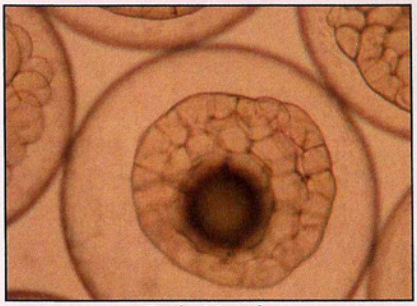
Four - cell stage



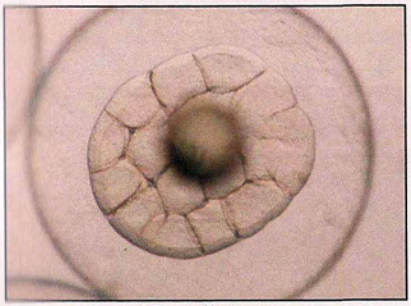
64 - cell stage



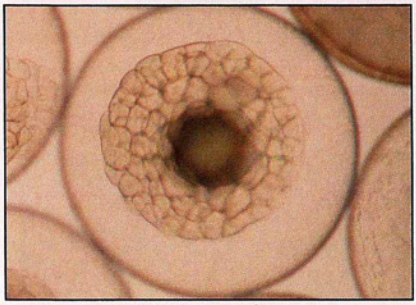
Eight - cell stage



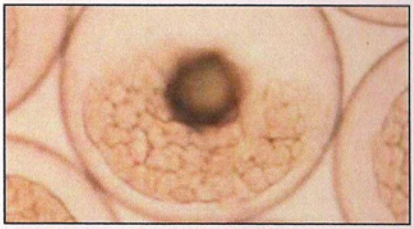
Early Morula



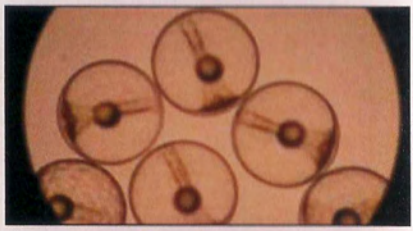
16 - cell stage



Late Morula

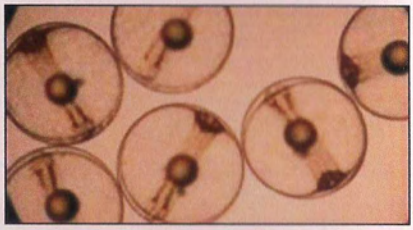


Early Blastula

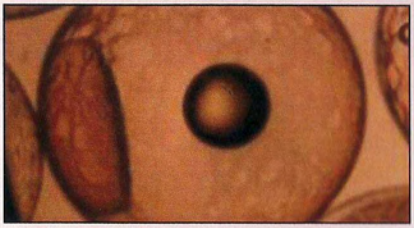


Early Gastrula

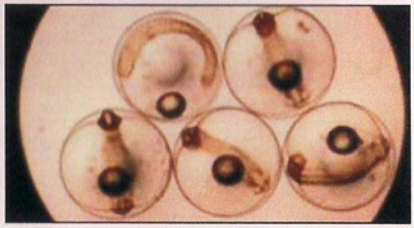
High



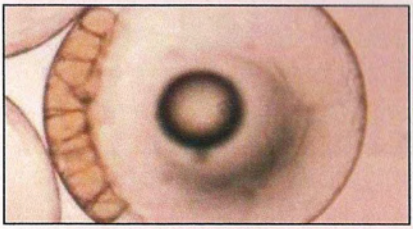
Mid Gastrula



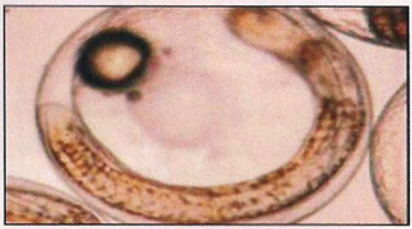
Dome



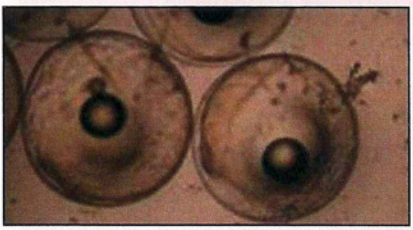
Late Gastrula



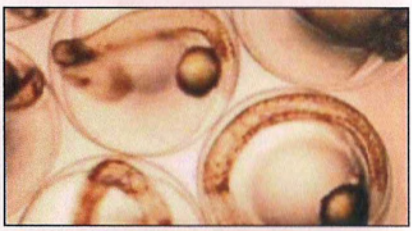
Oblong



Bud



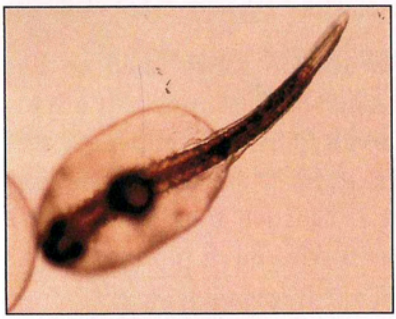
Epiboly 30%



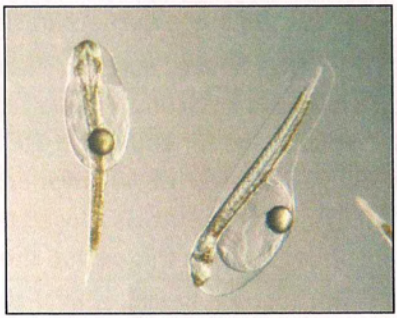
Segmentation

High-pec

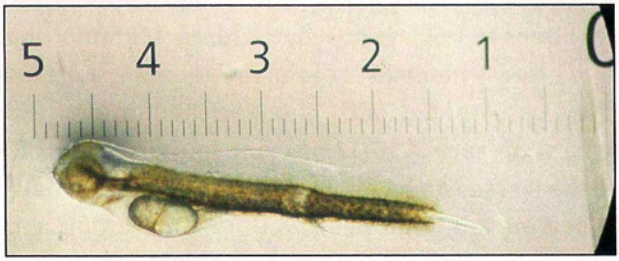
Hatching in progress



Newly hatched larva



Larvae-12 hour post hatch



2dph

5. Larviculture

Newly hatched larvae have to be checked to assess their viability and condition prior to stocking in the larviculture tanks. At least 10 to 20 fish larvae have to be observed under the microscope for the following:-

- shape and dimensions
- deformities, erosions and abnormalities
- appearance of internal organs
- pigmentation
- absence of external parasites

The larvae hatched in the incubation tanks need to be distributed in larviculture tanks to have minimal stocking density of 5 to 10 larvae/ litre for cobia and 10 - 20 larvae / litre for pompano. Care should be taken to avoid any mechanical stress or damage. Soon after hatching, the mouth is closed and the digestive tract is not fully developed. During this period the larvae survive on its reserves in the yolk sac.

Larviculture of cobia

Newly hatched larvae of cobia normally measures 3.4 mm size. Larval mouth opens at 3-5 days post hatch (dph). Metamorphosis starts from 9-11 dph. Newly hatched cobia larvae generally start feeding at 3 dph and they can be fed with the enriched rotifer (*Brachionus rotundiformis*) at the rate of 10-12 nos / ml, four times a day till 10 dph. From 8 dph, the larvae can be fed with enriched *Artemia* nauplii at the rate of 1-3 nos / ml, 2-3 times per day. During the rotifer and *Artemia* feeding stage, green water technique can be used in the larviculture system with the microalgae *Nannocloropsis occulata* at the cell density of $1x10^5$ cells / ml. The weaning to artificial larval diets has to be started from 15- 18 dph. While weaning, formulated feed should be given 30 minutes prior to feeding with live feed. Size of the artificial feed has to be smaller than the mouth size of the fish. Continuous water exchange is required during weaning stage. Between 25-40 dph, the larvae are

highly cannibalistic and hence size-grading has to be undertaken at every four days interval. During this stage, the fry could be weaned totally to artificial diets. Larval rearing can be practised both intensively in tanks and extensively in tanks/ponds. The major factors affecting the growth and survival of larvae are nutrition, environmental conditions and handling stress. Since there is high demand for essential fatty acids (EFAs), enrichment protocols are needed for live-feeds. The water exchange can be practically nil till 7dph and it can be gradually increased from 10-100 % from 8 to 12 dph. The environmental conditions required during the larviculture period are DO > 5mg / l , NH_3 < 0.1mg / l, pH: 7.8–8.4, Salinity: 25-35 ppt, water temperature : 27-33° C.

Green water has to be maintained in appropriate densities in the larval tanks. While weaning the fish larvae from rotifers to artemia nauplii, co-feeding with rotifers has to be continued due to the presence of different size groups of larvae. The detail of weaning protocol is as follows.

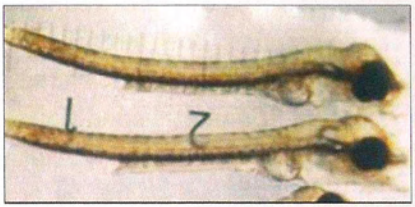
Age of Larvae (dph)	Size of Larvae (cm)	Size of Feed (µ)
18 – 19	2.3 – 2.6	100-200
20 – 23	2.5 – 3.5	300-500
23 – 30	3.5 – 8.0	500-800
31 onwards	> 8.0	800-1200

The juveniles measuring 10 cm length are ready for stocking in happas/ nursery tanks.

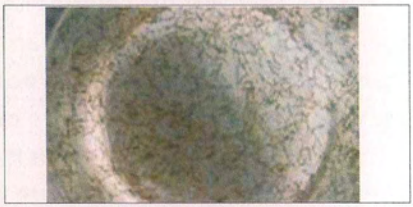
Nursery and grow-out rearing of cobia

Nursery phase of cobia can be carried out in happas or sea cages or indoor FRP / cement tanks. During nursery rearing, it is advisable to feed the juveniles with formulated feed of 1200 µ size which can be increased to 1800 µ size from 55 dph onwards. Once the juveniles reach a size of 15 gm, they are ready to stock in sea cages or land based ponds for grow-out farming.

Larval stages of cobia



3 dph

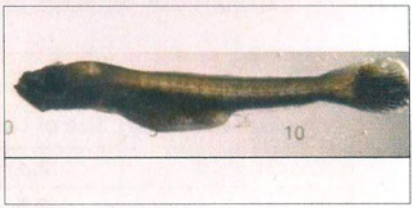


4 dph

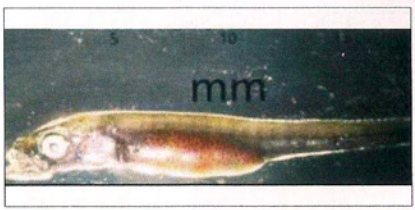
6 dph

10 dph

13 dph

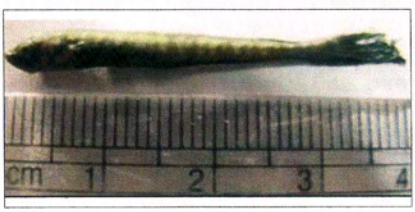


15 dph



20 dph

21 dph



25 dph

30 dph

Larviculture of Pompano

The newly hatched larvae are stocked at a density of 10000 larvae in FRP tanks of 2 m^3 capacity filled with 1.5 m^3 filtered seawater. The tanks are provided with mild aeration and green water at a cell density of 1 x10^5/ml. The mouth of the larva opens on 3dph and the mouth size is around 230 µ.

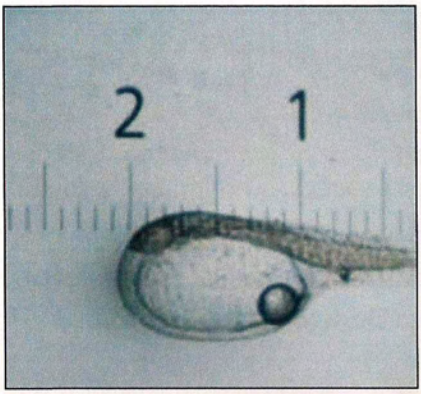
The larvae are fed from 3 dph to 10 dph with enriched rotifers at a density of 5-6 nos. per ml, wherever possible, wild collected copepods can also be added as supplements. Enriched *Artemia* nauplii are provided at a density of 1-2 nos. per ml from 8-19 dph. Weaning to larval inert feeds can be started from 15 dph. From 25 dph onwards, feeding can be entirely on larval inert feeds. The metamorphosis of the larvae starts from 18 dph and all the larvae metamorphose into juveniles by 25 dph. Critical stage of mortality would occur during 3-5 dph and subsequent mortalities are negligible. The water exchange can be practically nil till 7dph and it can be gradually increased from 10-100 % from 8 to 14 dph.

Nursery Rearing of Pompano

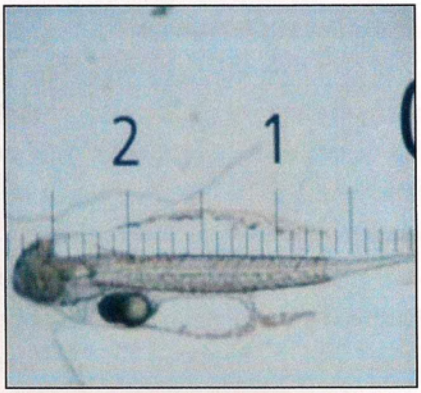
Nursery rearing could be initiated from 25 to 30 dph. At this stage, artificial feed of 800 µ size could be provided. Thereafter, fingerlings were fed with progressively higher size range of floating extruded larval feeds. Daily water exchange of 100% is advisable. Water quality parameters like salinity, temperature, pH, Oxygen level and ammonia are closely monitored during the entire larviculture period.

After 55dph, the fingerlings with size range from 1 to 1.5 inch size can be supplied to farmers for stocking in the happas / tanks for further nursery rearing and grow-out farming thereafter.

The pompano fingerlings can be reared at salinities as low as 5 ppt. At lower salinities from 10 to 15 ppt, they grow faster than in pure seawater.



Newly hatched larva

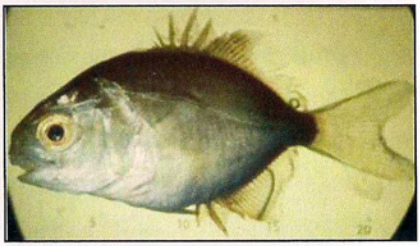


Larva on 2 dph

3 dph

13 dph

18 dph

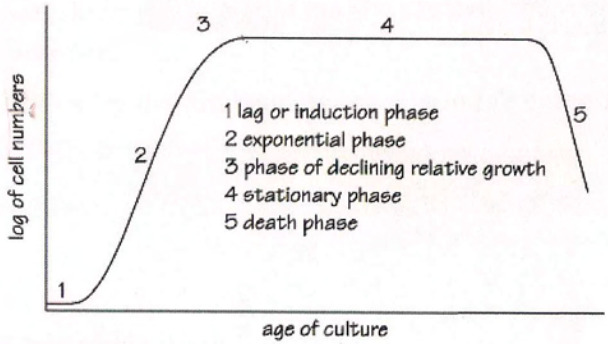


28 dph

6. Live feed culture

Microalgae are the important live feed required for larviculture of marine fin fishes. Algae like *Chlorella* sp., *Nannochloropsis* sp., *Tetraselmis* sp., *Dunaliella* sp., *Pavolova* sp. and *Isochrysis* sp. can be used as algal diet for growing the rotifers. The size, nutritive value, proliferation rate and digestibility of the algae are the critical factors for selecting the algae for the use in marine hatchery use.

Algal growth phases

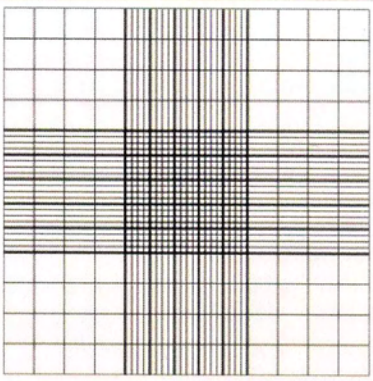


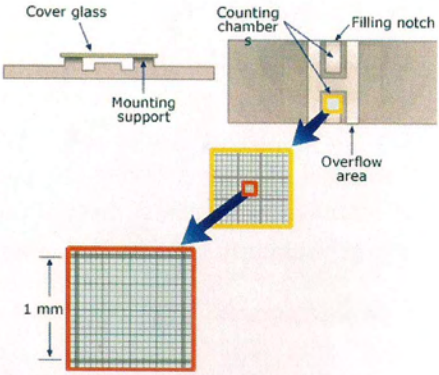
Nutrition required by Microalgae

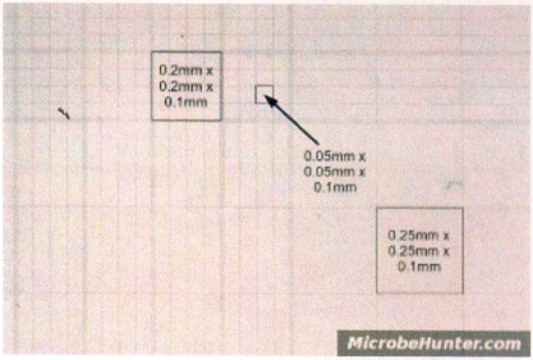
- Macro elements include nitrate and phosphate as prominent nutrients
- Micro elements are iron, molybdenum, copper, zinc, cobalt, B1 (thiamine), B12 (cyanocobalamin) and biotin.

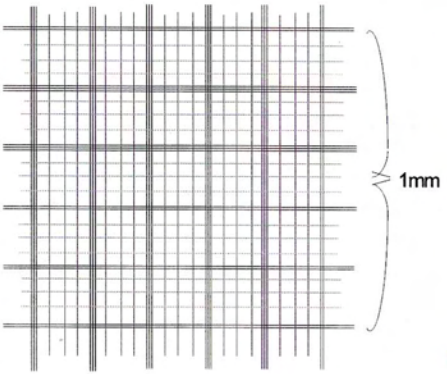
Measurement of cell density

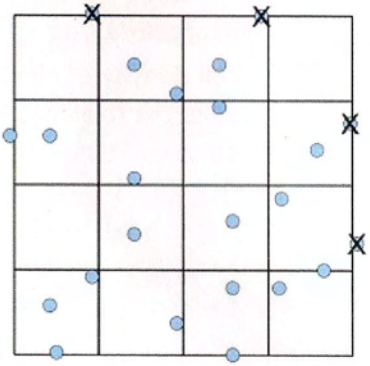
The cell density of algae is measured using haemocytometer. The ruled area of the haemocytometer consists of several, large, 1 x 1 mm (1 mm^2) squares. These are subdivided into 3 ways; 0.25 x 0.25 mm (0.0625 mm^2), 0.25 x 0.20 mm (0.05 mm^2) and 0.20 x 0.20 mm (0.04 mm^2). The central, 0.20 x 0.20 mm marked, 1 x 1 mm square is further subdivided into 0.05 x 0.05 mm (0.0025 mm^2) squares. The raised edges of the haemocytometer hold the cover slip 0.1 mm off the marked grid. This gives each square a defined volume.




Cover glass
Mounting support
Counting chambers
Filling notch
Overflow area
1 mm


0.2mm x
0.2mm x
0.1mm
0.05mm x
0.05mm x
0.1mm
0.25mm x
0.25mm x
0.1mm
MicrobeHunter.com


1mm



- The haemocytometer is a specialized microscope slide on which 2 grids have been engraved, in a central region that is 0.1 mm lower than the rest of the slide.
- Each grid comprises 25 large squares, each containing 16 smaller squares of area 1/400 mm^2.
- This creates a region of known volume (0.1 mm^3) when a special cover slip is correctly placed over the central region.
- 10µl of culture are then pipetted under the coverslip and cells counted in a proportion of the grid squares (count as many as is convenient).
- Multiplying the total number of cells in the entire grid by 10^4 gives the number of cells per ml.

Laboratory Scale media (for stock culture up to 2000 ml flask)

Walne Media		
$NaNO_3$	: 100 g	A-solution
Na_2EDTA	: 45 g	
$Na_2H_2PO_4 2H_2O$	: 20 g	
$FeCl_3 6H_2O$	: 1.3 g	
$MnCl_2$	: 0.36 g	
H_3BO_3	: 33.6 g	
Vitamin B1	: 0.1 g	B-solution
Vitamin B12	: 0.005 g	
$ZnCl_2$	: 2.1 g	C-solution Ratio 1:0.5:0.1
$CoCl_{12} 6H_2O$	: 2.0 g	
$(NH_4)Mo_7O_{24} 4H_2O$	: 0.9 g	
$CuSO_4 5H_2O$	: 2 g	

Algal Culture Management

Stage I - (Agar culture, test tube and Erlenmeyer 100 - 250 ml, 500 - 1000 ml)

- The turbidity of media is approximately zero/nil which can be achieved by filtering water with 5 µm and 1 µm cartridge filters and a UV filter.
- Salinity can be decreased (28-30 ppt) with the addition of 10 % distilled water. (V1N1 = V2N2)
- Use only laboratory grade media.
- Autoclaving
- Incubation has to be done at 19 - 23°C and 1 or 2 neon lamps with 40 watt each has to be provided above the culture tank.
- One or two drops starter or ratio of 1 to 5 or 1 to 10 has to be added.

Stage II (bottle culture 1000 ml)

- The turbidity of media is approximately zero/nil which can be achieved by filtering water with 5 µ and 1 µ cartridge filters and a UV filter.
- Salinity has to be maintained at 30 - 32 ppt
- Laboratory grade fertilizer can be used (ratio 1:0.5:0.1)
- Sterilization with chlorine 10 ppm has to be done and it can be neutralized with ≤ 5 ppm of sodium thiosulfate.
- Incubation at 24 °C with two neon lamps with 40 watt each is required.
- Starter algae can be added with ratio of 1 to 2 or 1 to 5
- Incubation of 21 to 25 days is required.
- Frequent mixing of the flask is required

Stage III (Carboy culture 20 liter)

- Salinity of media can be 31-32 ppt
- Use laboratory grade fertilizer (ratio 1:0.5:1)
- Sterilization with 10 ppm chlorine and neutralization with

sodium thiosulfate (5 ppm)

- Keep at the temperature 24 °C with two neon lamps (40 watt each)
- Put algae starter with ratio of 1 to 7
- Supply of CO_2 using aeration
- Incubate for 5 to 7 days

Stage IV (Intermediate Culture in an aquarium with volume of 100 liter)

In outdoor

- Salinity 32-33 ppt
- Use commercial grade fertilizer
- Chlorine sterilization at 5 -10 ppm
- Use filter bag to filter culture water
- Aeration
- Temperature 29-30 °C
- Expose in direct sun light
- Incubate for 5 to 7 days

Stage V (Intermediate fiber glass culture in 1 m³ volume)

In outdoor

- Salinity of media: 32 - 33 ppt
- Use commercial grade fertilizer
- Sterilize with chlorine at the dosage of 10 ppm
- Use filter bag to filter culture water
- Aeration
- Expose in direct sun light
- Incubate for 5 to 7 days

MASS CULTURE OF ALGAE

- To fulfill the volume of feed needed, microalgae cultured in large concrete / FRP tanks starting from 2 ton, 5 ton, 10 ton, and 20 ton are required.

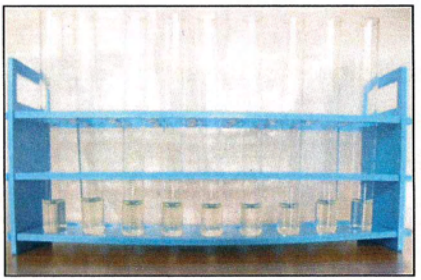
- The objective of mass plankton culture is to fulfil the necessity of zooplankton feed (rotifer) and to supply into the larva tank as the enrichment of zooplankton feed, the support of water quality and shading effect.
- The tank culture of algae has to be equipped with aeration on some points for fertilizer turbulence.
- The culture of microalgae (inoculums) has to be taken from the intermediate laboratory culture and then cultured in all the tanks gradually until the sufficient algal mass is achieved.
- It is necessary to sterilize the water using chlorine 10 ppm for 24 hours, then to neutralized by using Sodium Thiosulphate at 5 ppm. To enrich fertilizers, several agricultural fertilizers can be used as shown in the Table below

Fertilizer	Concentration (ppm) / tonns
Urea	40-60
Ammonium sulphate	20-40
Ammonium phosphate	20-30
EDTA	1-5
$FeCl_3$	1

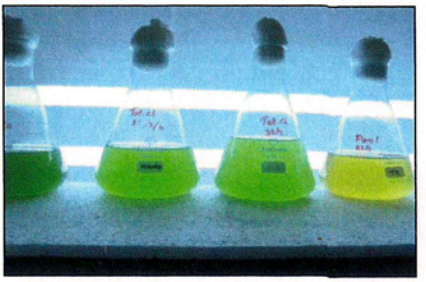
(or)	
Ammonium sulphate	100 g
Ammonium phosphate	10 g
Urea	10 g
Ratio - (1:1:1)	

- If the sunlight is normal, the algae will grow and will be ready for use after 4-5 days, with the density of 12-15 million cells/ml.
- *Microalgae* should be harvested before it reaches the peak of growth or stationary phase.
- Algae can be directly pumped to the destination tank such as algae seed tank, rotifer culture tank, copepod tank and larval rearing tank.
- Around 10 % of the microalgae in a mass culture tank can be retained for re-culture by adding filtered seawater and fertilizer. This method can be employed for 2-3 times.
- After that microalgae in the culture tank is totally harvested and the tank needs to be cleaned to prepare for a new culture.
- For a medium size fish hatchery, several tanks are required to culture phytoplankton so that it can be harvested every day by turns.
- The following water quality parameters are considered optimum for mass culture of algae

No.	Parameters	Range
1.	Salinity	30 – 33 ppt
2.	Temperature	26 – 31 ° C
3.	Light	12 L : 12D
4.	pH	8.1 – 8.3
5.	Dissolved Oxygen	4.5 – 6.5 ml / lit

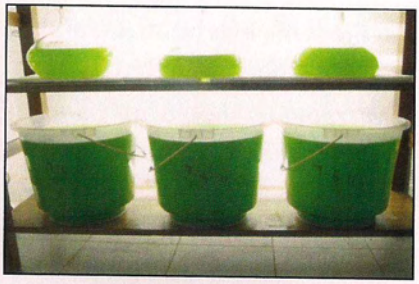


Algal culture in test tubes



Algal culture in conical flasks

Algal culture in flasks



Algal culture in carboys

Copepod culture

Copepods have almost become inevitable because they are the only acceptably sized prey for small larvae of many marine fin fish species and the only type of live feed that will support the altricial type of larvae. Copepod nauplii offer a diverse size spectra and nutritious prey that can meet the specialized needs of small fast growing fish larvae. Over the past few years, several articles have been published and many conferences were dedicated to discussions of copepod culture and the important role that copepods can play as live feed for marine finfish larviculture.

Advantages of Copepods

The three main copepod orders *viz.*, Calanoida, Harpacticoida and Cyclopoida have been investigated for their suitability as feeds for larval and juvenile fish. While each copepod order has its advantages and disadvantages, it is generally agreed that the following are the benefits of using copepods for larviculture.

(i) Copepods have a larger size range from first nauplii to adult copepodites and offer good size ranges for the entire hatchery phases for certain species of finfish

(ii) They have superior nutritive value in comparison to rotifers and *Artemia*

(iii) Copepod nauplii may be more easily and completely digested than either rotifers or *Artemia*

(iv) Copepods are natural sources of antioxidant astaxanthin and Vitamins C and E

(v) The movement of copepods and their nauplii triggers the feeding responses in fish larvae. The 'jerking' swimming action of most copepod nauplii and adults is an important stimulus for initiating feeding by fish larvae

(vi) Use of copepods in larval fish diets have been associated with a decrease in fish malpigmentation and deformity rates

Euterpina acutifrons* - *Harpacticoid copepod

- It is being cultured at our centre.
- It is free living and benthic organism.
- Inhabit sediments occupying spaces between sand particles, burrowing into sediment or living on sediment. High tolerance to a wide range of environmental conditions
- Harpacticoid copepods are favoured over calanoids, since harpacticoids, as a result of their benthic habitat, can be reared at much higher densities. However, their benthic nature also makes mass culture difficult, since large surface areas must be provided.
- Harpacticoids are primarily detritivorous, benthic grazers, efficiently utilizing various food sources such as micro algae, algal bio-film and diatoms.
- Harpacticoids are smaller and range in size from 40µ (Nauplii -1) to 800µ (adult), short life cycles (7-29 days) and having planktonic naupliar stages.

Life cycle

- The male deposits a sac containing viable sperm called a spermatophore near the genital aperture of the female.
- Harpacticoids have their eggs contained within one or two egg sacs which remains attached to the female genital segment until they hatch.

- Egg production is measured as number of eggs per female per day.
- Fecundity ranges from 18 to 20 eggs.
- Fertilized eggs pass into the water or into an egg sac.
- The egg is spherical and protected by a chitinous envelope.
- The larvae that hatched from copepod eggs, the nauplius) develops through 6 moults before passing into the copepodite stage where they display the general adult factures.

Isolation, Identification and culture

- Collection from the wild – filter with particular mesh size (100 µ to 500 µ)
- Identify the individuals using microscope and identification keys
- Stock 100 no. of individuals (1:1 M/F) to 50 ml test tube
- Add microalgae as feed (5 ml / 2 days once)
- Provide light period 12L / 12 D; Salinity: 27 to 35; Temperature: 28 to 31°C
- Generation time – 7 days
- Harvest nauplii / copepodite (harvest continuously for 3 months)

Intermediate culture

- Nauplii (1000 nos) transfer to 1000 ml beaker
- Add microalge as feed (25 ml / 2 days)
- Provide light period 12L / 12 D; Salinity: 27 to 35; Temperature: 28 to 31°C
- Generation time – 7 days
- Collect nauplii / copepodite

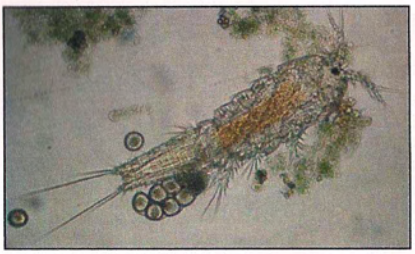
Mass culture

- Transfer nauplii to 1 to 2 tonne tank
- Add microalgae as feed (500 litres algae / week)
- Provide light period 12L / 12 D; Salinity: 27 to 35; Temperature: 28 to 31°C

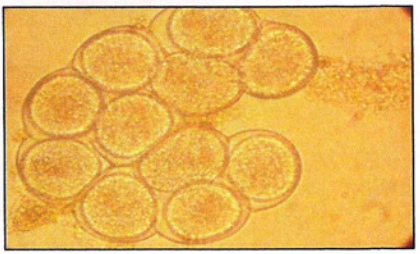
- Generation time – 7 days
- Harvest nauplii / copepodite (3-5 nos / ml)
- Total drain and harvest the tank after 1 month

Culture Protocol

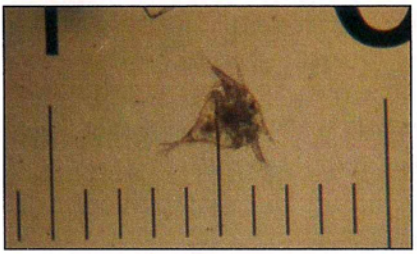
- Population counts should be done weekly for feeding adjustments.
- Count algae concentrations and compute required feed volumes to be added.
- Siphon detritus from tank bottom daily.
- Remove sufficient water volume to allow addition of new feed volume(s), while maintaining 200 litres total volume. As the population matures this volume can be increased to 300 litres. Adjust feed computations accordingly.
- Use of 40 μ sieve while cleaning of bottom of the culture tank will also remove copepodites, nauplii, and eggs. The bucket containing the wastes is left undisturbed for 10 minutes to allow settling. The copepodites and larval stages are then attracted to the surface of water using light and decanted back into the tank.



Adult berried copepod



Copepod eggs – a close up view



Nauplii stage of copepod

Rotifer culture

Rotifers are the smaller size zooplanktons widely used in marine fin fish hatchery operations. The marine fin fish larvae initially feeds on the such smaller size zooplanktons and hence suitable size of rotifers need to be cultured in mass to feed the fish larvae. The important criteria for selecting the rotifer depends on the mouth size of the fish larvae, digestibility, nutritive value of the rotifer and easy for culture and proliferation. Marine and brackish water rotifer species can be artificially propagated in seawater and more popular rotifer species used for marine fin fish hatcheries are *Brachionus plicatilis* and *Brachionus rotundiformis.*

Based on the length of lorica, *Brachionus* is separated into 3 strains,

B. plicatilis as L type (large) with long of lorica 200–360 µ

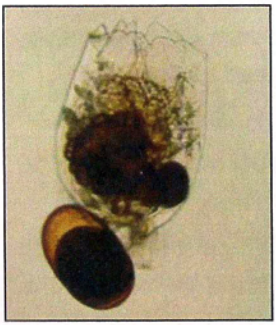
B. rotundiformis as S type (small) with long of lorica 150–220 µ

B. rotundiformis as SS type (super small) with long of lorica 70–160 µ

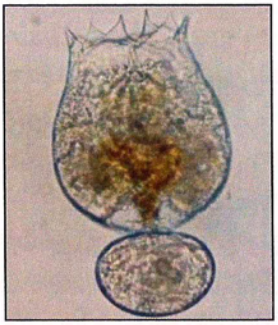
WHY DOES WE CHOICE ROTIFER AS LIVE FEED?

1. Small body size and round shape
2. Slow swimming speed and habit of staying suspended in the water column
3. Easily enriched with external nutrients resources
4. High reproduction rate and high density cultures
5. Very rich in low molecular weight water soluble proteins
6. Contain a broad spectrum of digestive enzymes such as proteases, peptidases, amylases, lipases and even celluloses

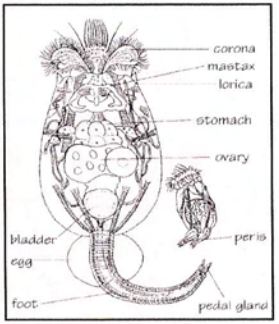
Rotifer's body is symmetrical and appears like a cup. Body of rotifer can be divided into three main parts, as head, body and leg or tail. Head of rotifer consists six *lorica* spine and 1 pair of longest spine on centre.



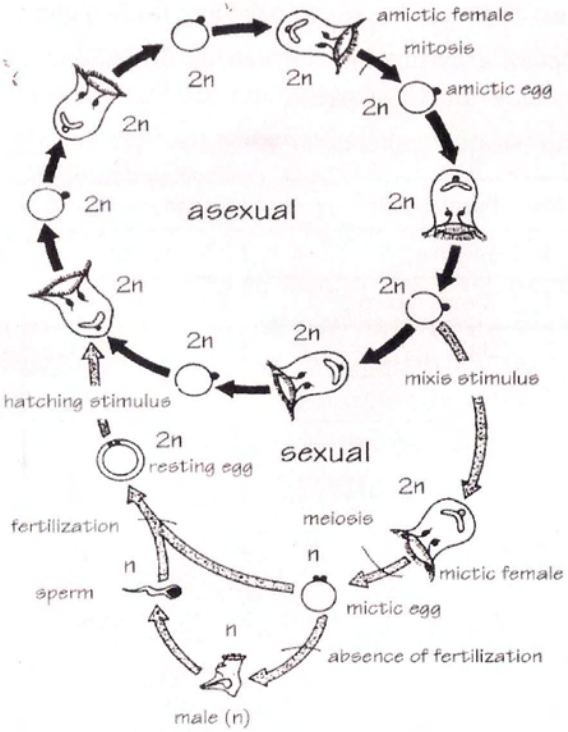
Brachionus plicatilis



Brachionus rotundiformis



Morphology



Pure culture of Rotifer

- The rotifers are incubated in 500 ml Erlenmeyer containing sterile sea water. The culture condition should be equipped with aeration to supply oxygen and flourescent lamp.

- Microalgae such as green algae (*Chlorella* sp, *Tetraselmis* sp, *Dunaliella* sp., *Isocrysis* sp) can be used as feed for rotifer.

Mass culture of Rotifer

- Outdoor mass culture can be carried out successfully in 1 to 10 tonne capacity tanks.
- Phytoplankton has to be added to rotifer culture tank with density of 3-4 million cell/ml for starting the rotifer culture. Rotifers have to be inoculated into tank with initial density of 50-100 rotifers/ml
- The next day, algae has to be added into rotifer culture tank
- Practically, growth of rotifer can be identified by change of water colour into transparent
- Optimum water quality parameters are

No.	Parameters	Range
1.	Salinity	27 – 33 ppt
2.	Temperature	26 – 31 ° C
3.	Light	2,000 – 3,000 lux
4.	pH	7.5 – 8.3
5.	Dissolved Oxygen	3 – 6.5 ppm

Continuous Culture

- Rotifers can be harvested after a period of 4-5 days, by flowing out culture water through a plastic hosepipe into a rotifer sieving bag with mesh size of 60 µ.
- Collected rotifers can be transferred into bucket for enrichment with different types of enrichment media.
- Every day, around 30% volume of rotifer culture tank has to be harvested from the total volume of tank
- After repeated use of 3 weeks, the rotifer culture tank should be totally cleaned for fresh culture

Rotifer enrichment

- Rotifers can be enriched with Highly Unsaturated Fatty Acids (HUFA's) which contains high levels of the essential Omega-3 fatty acids, Eicosapentaenoic acid (EPA) Deicosahexaenoic acid (DHA).
- HUFAs are important to maintain fluidity of blood in blood vessel under low temperature condition for the fish larvae
- Especially DHA, an essential fatty acid that accumulates in the brain of fish during early development and increases neural function
- Feeding with DHA-enriched diet at an early stage of fish larvae has been successful in improving pigmentation
- EPA deficiency results in poor vitality, poor growth, low survival and death to simple stress (shock).

Enrichment methods

- Indirect method through feed medium like *Nannochloropsis* and ω – yeast. Rotifers in each culture tank can be fed with concentrated microalgae of *Nannochloropsis* sp. ($1x10^5$ cells/ml) every 8 hrs and yeast once in a day at morning.
- Direct method with emulsified oil: 100 million rotifers with fish oil (5 ml), raw egg yolk (2 ml) and tap water (100–200 ml).

Enrichment with Scot emulsion

- Harvested rotifer has to be transferred into a 35 litres tub containing 3/4 part of *Nannochloropsis* medium and 1/4 part of rotifer (600 to 800 nos / ml)
- 1-5 ml Scots emulsion oil can be mixed with freshwater and stirred well.
- This emulsion can be added into the above-tub.
- Strong aeration has to be provided for 2 hours.
- Harvest rotifers and feed the fish larvae immediately.

- **INVE A1 DHA / INVE Sparkle and INVE SELCO (Self Emulsifying Liquid Concentrates)**
  - Four numbers of 2 ton tanks of rotifers are harvested daily (four days after stocking) at a density of 600-800 rotifers/ml.
  - Enrichment is done in buckets containing a volume of 40 litres with a density of 800-1000 rotifers/ml. Approximately 10 litres of *N. oculata* ($1.0x10^2$ cells per ml) are added.
  - Buckets receive aeration to maintain DO levels above saturation.
  - Enrichment media (INVE DHA selco) at doses of 0.3 g per $1x10^5$ rotifers were added and rotifers are allowed to feed for 4-6 h at 27-28 °C.
  - Once enrichment is completed, rotifers are filtered to remove residual enrichment diet and placed into a clean bucket containing a final volume of 35 litres.
  - Enriched rotifers at a density of 10-12 nos. per ml were fed to the fish larvae.

Artemia nauplii

Having a larger size than rotifers, the nauplii of brine shrimp *Artemia* are used as the second live food to fed fish larvae. Commercially available *Artemia* cysts are purchased and hatched whenever required. The first *Artemia* larval form is the nauplii, which are smaller in size and richest in yolk, and followed by larger size metanauplii, whose nutritional value has to be boosted by feeding them with special enrichment diets 12 to 24 hours before feeding them to the fish larvae.

Two additional parameters characterize the *Artemia* batches: the number of cyst per gram and their hatching rate (the number of nauplii produced per gram of cysts). The best strains can give about 200 000 - 250 000 nauplii per gram of cyst hatched, with a hatching rate close to 95%. In a hatchery the use of good quality cysts allows a synchronization of the production cycle on a 24-h period, with the harvest of freshly hatched nauplii coinciding with the start of the

incubation of new batches.

Disinfection and decapsulation of brine shrimp cysts

Artemia cyst shells are usually contaminated with bacteria, spores of fungi and other microorganisms. Fish larvae can be infected when untreated empty shells, unhatched cysts or cyst hatching medium residues that are introduced into the larval rearing tank. Therefore, cysts should be disinfected before incubation. This process also improves hatching by reducing the bacterial load of the hatching medium. Disinfection is done by keeping the cysts for a few minutes in a hypochlorite solution at a maximum density of 50 g/litre. This product is easily available as commercial grade bleach. The duration of the treatment varies according to the active chlorine concentration of the disinfecting solution.

The following example shows how to disinfect one kg of cysts in a 200 ppm hypochlorite solution obtained from a household bleach with 5% active chlorine:

- One kg of cysts needs 20 l of fresh water for the disinfecting solution.
- If this solution is going to be used for a 20 minutes bath you will need 20 l x 200 mg/l = 4 000 mg = 4 g active chlorine.
- The quantity of 5% bleach required to give 4 g active chlorine is: (1 000/50) x 4 = 80 ml. Pour 80 ml of 5% bleach in 20 litres of fresh water.
- Add one kg of cysts; place an airstone for continuous aeration to keep cysts in suspension, and keep the cyst in the solution for 20 minutes.
- Harvest cysts on a sieve (125 µ mesh size) and rinse thoroughly with plenty of tap water.
- Transfer the rinsed cysts to the incubation tank.

A more effective way to obtain completely contaminant-free cysts is decapsulation, which implies the elimination of the cyst's thick external layer, the chorion, by chemical oxidation. This process, which

requires greater attention, has additional advantages. As they spend less energy to hatch after the removal of the chorion, the hatching nauplii have better nutritional value. Moreover, fish do not risk suffocating by gulping empty or unhatched cysts offered together with the nauplii, as it may happen when using disinfected cysts. The decapsulation process consists in four steps: hydration, treatment in a chlorine solution, washing and deactivation of the residual chlorine. The example described below refers to the decapsulation procedure of one kg of cysts. The hydration, a necessary step as the complete removal of the chorion may only happen when cysts are spherical, proceeds as follows:

- Water volume required: around 6 litres per kg (maximum amount: 200 g/l); both fresh and sea water can be used; water temperature should be between 20-25°C;duration: 45 minutes
- Aeration: sufficiently strong to keep cysts in constant suspension; use an open end pipe in a 10 litres bucket.
- Collect the hydrated cysts on a sieve and treat them immediately with the decapsulation solution.

The decapsulation solution requires a source of hypochlorite, usually liquid bleach and an alkaline product to increase pH level of the decapsulation solution above pH10. Usually technical grade caustic soda (sodium hydroxide) is utilized. The first product is added at 0.5 g active chlorine per gram of cysts, and the second as 0.15 g of sodium hydroxide per gram of cysts. For hydrated cysts the procedure is as follows (figures refer to one kg of cysts):

- Prepare 0.5 g Cl x 1000 g cysts = 500 g of active chlorine, equal to 333 l of a 15% bleach.
- Prepare 0.15 g NaOH x 1 000 g cysts = 150 g of NaOH, equal to 0.375 l of a 40% NaOH solution.
- Put the bleach and NaOH in a suitable container (e.g.: a 20 litres plastic bucket) and fill with seawater to 14 litres (14 – 3.33 – 0.375 = about 10.3 litres of seawater) provide a strong aeration and eventually if available add antifoam.

- Place the hydrated cysts in the bucket; control the temperature: it should remain within 25°-30°C. In case of higher temperatures, add ice to prevent that it reaches 40°C which are lethal for the cysts.
- Verify cyst colour changes. The change in cyst colour confirms that decapsulation is in progress.
- The cyst colour shifts from dark brown to grey and finally to orange, which is the colour of the nauplius body seen by transparency through its outer cuticular membrane, left exposed by the dissolution of the chorion. The process usually lasts 5 to 15 minutes.
- Using a pipette or a graduated cylinder, check floatability: non decapsulated cysts will float and decapsulated cysts will sink; as soon as all cysts have turned orange, stop the process by harvesting them on a sieve and rinse thoroughly with plenty of tap water and rinse well until no more chlorine smell is noticed.
- The residual hypochlorite adsorbed by the decapsulated cysts has to be neutralised by dipping them in a 0.1% solution of sodium thiosulfate ($Na_2S_2O_3 . 5H_2O$) for 5 minutes; then, after a final rinsing, they are transferred to the incubation tank.

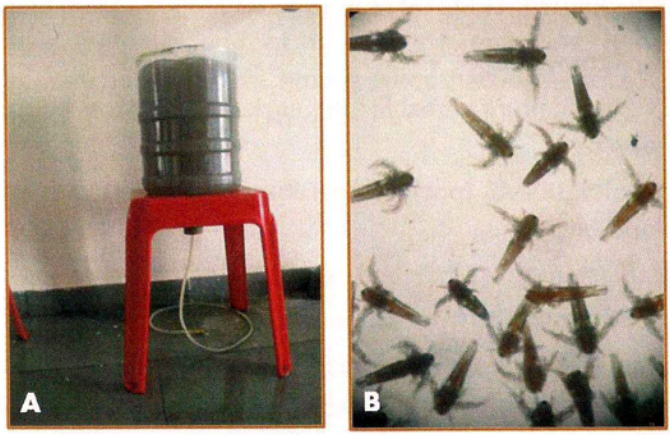


Figure 1. A small scale *Artemia* cyst hasting system and B. Microscopy observation of hatched *Artemia nauplii*

Counting and evaluating Artemia nauplii

- Take a 10-ml sample of the population to be estimated.
- Dilute the nauplii concentration by adding 90 ml of sea water to obtain a total sample volume of 100 ml.
- Take three sub-samples with a 1-ml pipette, avoiding to suck air bubbles.
- Transfer each sub-sample to a Petri dish.
- Add a few drops of the fixative staining solution Lugol to each of the Petri dishes and wait until all nauplii are immobile and deeply stained.
- Add water so as to distribute the nauplii over the whole surface of the three Petri dishes.
- Put each Petri dish on a grid and count the nauplii present in each 1 ml sub-sample.
- Calculate the average number of nauplii per ml by dividing the sum of the three counts by three.
- The total nauplii density per litre is given by the average number of nauplii per ml multiplied by 10.
- To give the number per undiluted ml and finally by 1.000 to get the final density per liter.

***Artemia* enrichment**

- Prepare culture vessel for the enrichment process using fresh seawater.
- Salinity 35 - 45 (ppt); temperature: 27 - 28 °C and vigorous aeration.
- Harvest the newly hatched nauplii.
- Gently rinse with fresh water with suitable filter.
- Transfer to a clean culture vessel.
- Stock at the rate of 8,000 -10,000 newly hatched brine shrimp per liter

- Do not feed nauplii at this time - they are absorbing their attached yolk sac.
- Approximately 8 to 10 hours from time of transfer to clean culture vessel, the nauplii will have molted into the Instar II feeding stage.
- Add SELCO 0.2 g per 1,00,000 Artemia nauplii per liter.
- Ensure that the aeration is vigorously mixing the water column.
- After approximately 12 hours, the intestinal tract of the nauplii should be fully enriched with SELCO.
- Harvest enriched nauplii and feed immediately maintaining a nauplii concentration of 2-3 nos. per ml.
- Unfed enriched nauplii can be stored in the refrigerator for later feedings.

Enrichment using Instar-II-nauplii:

- Add 0.6 g (in a minimum of 2 rations during 0 hrs – 12 hrs) of A1 DHA SELCO per liter of seawater containing up to 3,00,000 *Artemia* nauplii, in the enrichment tank. Enrich for a period of 24 hours while maintaining DO at 4 ppm and pH at 7.5-8.5.
- Prepare the enrichment meal as specified by the manufacturer, and make sure to prepare a new enrichment emulsion for each meal. At the end of the enrichment time harvest the metanauplii as usual, rinsing them thoroughly with seawater until no oily emulsion is noticed in the outflowing water.

7. Diseases and health management

Introduction

Sustainable aquaculture production can only occur when fish are healthy and free from disease. Fish disease management is a combination of preventing the onset of disease and measures to reduce losses from disease when it occurs. Fish cultured in floating cages become particularly susceptible to disease when various environmental parameters such as temperature, salinity, dissolved oxygen and suspended particles fluctuate suddenly or widely, or following rough , although often unavoidable, handling operation. Once conditions suitable for pathological changes develop, progress to disease in the warm water environment is rapid. Early detection of behavioral changes and clinical signs in the cultured animals are critical for proper diagnosis of the disease.

Disease rarely results from simple contact between the fish and a potential pathogen. Environmental problems, such as poor water quality, or other stressors often contribute to the outbreak of disease.

What Is Fish Health Management?

Fish health management is a term used in aquaculture to describe management practices which are designed to prevent fish disease. Once fish get sick it can be difficult to salvage them.

Successful fish health management begins with prevention of disease rather than treatment. Prevention of fish disease is accomplished through good water quality management, nutrition, and sanitation. Without this foundation it is impossible to prevent outbreaks of opportunistic diseases. The fish is constantly bathed in potential pathogens, including bacteria, fungi, and parasites. Even use of sterilization technology (i.e., ultraviolet sterilizers, ozonation) does not eliminate all potential pathogens from the environment. Suboptimal water quality, poor nutrition, or immune system suppression generally associated with stressful conditions allow these potential pathogens to cause disease.

Predisposing factors

Fish stocks living under stressful conditions become less able to defend against a pathogen and hence will become sick more readily. Fish that are well cared for generally do not become sick even in the presence of a pathogen. The most common error in fish husbandry is overstocking. This leads to problems such as:

- Fish to fish aggression
- Increased fish and feed wastes
- Ease of disease spread,
- Increased concentration of pathogens
- Resultant poor water quality

High fish density, stress, and ease of transmission increase susceptibility of the fish population to diseases and parasites. In marine aquaculture, diseases present in wild fish can infect cultured fish and spread rapidly through the population

Types of Fish Diseases

There are two broad categories of disease that affect fish, infectious and non-infectious diseases. Infectious diseases are caused by pathogenic organisms present in the environment or carried by other fish. In contrast, non-infectious diseases are caused by environmental problems, nutritional deficiencies, or genetic anomalies; they are not contagious and usually cannot be cured by medications.

- **Infectious diseases**. Infectious diseases are broadly categorized as parasitic, bacterial, viral, or fungal diseases.

Common Diseases of Cobia (Rachycentron canadum)

S.No	Bacterial disease	Causative organism
1	Pasteurellosis	*Photobacterium damsella* sub sp *pisicida*
2	Streptococcosis	*S. iniae*
3	Vibriosis	*V.anguillarum*
4	Bacterial enteritis	*V.alginolyticus*
5	Mycobacterium infection	MY. Sp 2nd *Aeromonas hydrophila*
6.	Viral disease Lymphocystis	Irido virus

Common Diseases of Pompano (Trachinotus blochii)

S.No	Disease	Causative agent
1	White spot disease	Ciliate protozoan,Cryptocaryon irritans
2	Cardiac myxosporidiosis	Myxosporidian protozoan,*Henneguya*sp
3	Monogenetic trematode infestation	*Bicotylophora trachinoti*- gills Benedenia sp- body
4	Fatty degeneration	Dietary deficiency- protein
5	Parasitic dermatitis (infestation)	Sea lice (*Calligus elongatus*)

Common Diseases of Ornamental fishes

S.No	Disease	Causative agent
1	Red pest	Gram negative bacteria
2	Fin Rot	Gram negative bacteria
3	Fish tuberculosis	*Mycobacterium* spp.
4	External Gas Bubble disease	Various causesCommonly caused by excess gas in the system, brought about by super-saturation of gas in high pressure water mains

- **Vibriosis** is a bacterial disease causing significant losses of fish in marine fish farms. Cobia, Grouper, seabream, snapper and pompano species are affected. Vibriosis accounts for an estimated two-thirds of disease reported in grouper species. Vibriosis results in severe skin, muscle, fin, eye and internal organ damage of fish. Diagnosis of the disease requires bacteriological culture of kidney, spleen, skin or eye lesions.

Affected Fish	Disease name	Treatment
Cobia	1.Vibriosis	• Administration of antibiotics mixed in the feed- 2wks • Oxytetracyclin - @ 100mg/kg biomass/day
	2.Enterobacteriaceae	• Potentiated sulphonamides- @70mg/kg biomass/day i/m
Pompano	1.Caligus infestation	• Fresh water dipping for 3-5min • Bathing fish with hydrogen peroxide (350 500 mg/litre for 20 min) will remove mobile sea lice from fish. • Ceftriaxone 10mg/ kg biomass/day i/m (If secondary bacterial infection identified)

- **Non-infectious diseases**: Non-infectious diseases can be broadly categorized as environmental, nutritional, or genetic.
- A hygienic fish culture environment is essential to the health and productivity of farming operations. The reasons for this include:
- Disease risks are increased in poor and polluted environments.
- Quality of the product depends on clean and healthy environments.

The culture environment incorporates the following components

- Physical farm infrastructure e.g. fish cages, floats, nets, and utensils.
- Water quality e.g. dissolved oxygen and microbial contamination.
- Seabed sediments e.g. solid wastes measured as carbon, nitrogen and phosphorus.
- Introduced chemicals e.g. antibiotics, metals and pesticides.

Farm hygiene is vital to maintaining fish health. It involves routine activities carried out by the farmer to ensure the following:

- Removal of biofouling from net/pens.
- Cleaning of utensils and equipment used to handle or feed fish.
- Water quality testing and correction of poor water quality includes the following:
- Measure dissolved oxygen and water chemistry values e.g. salinity, temperature, pH, ammonia, nitrite and nitrates.
- Measure bacterial counts e.g. *Vibrio* spp. counts of the water
- Aeration to maintain dissolved oxygen
- Cleaning of the farm seabed and fallowing or rotation of sites
- Minimising organic pollution from fish wastes and feed wastes

Preventive measures

- Preventing the introduction of pathogens
- Maintenance of good water quality
- Avoidance or reduction of environmental stressors
- Adequate nutrition
- Isolation of cultured animals from feral stocks
- Immunization

Three steps to solve a disease problem

- Determining that a problem exists.
- Identifying the cause of the disease or source of the distress
- Successfully curing the fish and eliminating the disease or cause of distress.

Pictures:

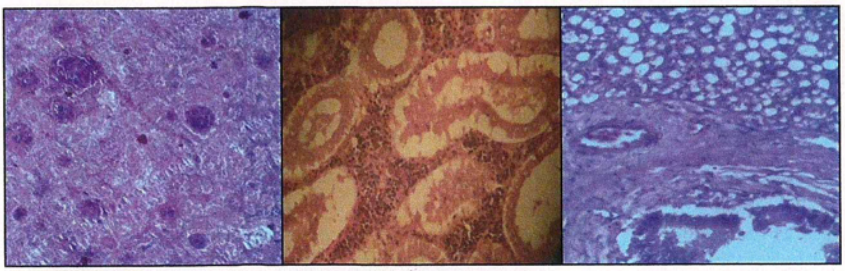
1) Fingerlings affected with Vibriosis

Eye: Bilateral exopthalmus

Stomach: Haemorrhagic

Haemorrhagic dermatitis & Septicaemia

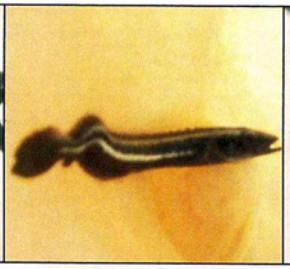
2) Histopathology :



Spleen:Liquefactive necrosis-H&E ***Kidney : Acute tubular necrosis H&E*** ***Liver : Fatty degeneration -H&E***

3) Genetic anomalies

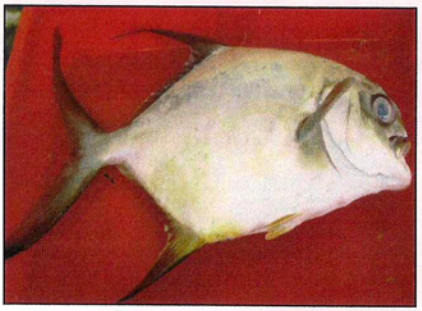
Cobia larvae with malformed upper jaw



Cobia larvae with bent vertebral column

Cobia with incomplete formation of jaws

4) Pompano affected with sea lice (*Calligus elongatus*) infestation



Pompano with caligus infection

Caligus lice